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(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11) Publication number:

**0 578 231 A1**

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 93110883.1

(51) Int. Cl.<sup>5</sup>: A61K 47/48, A61K 31/42

(22) Date of filing: 07.07.93

(30) Priority: 08.07.92 SI 9200139

(43) Date of publication of application:  
12.01.94 Bulletin 94/02

(64) Designated Contracting States:  
AT BE CH DE DK ES FR GB IT LI NL SE

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(54) Inclusion complexes of clavulanic acid and of alkali salts thereof with beta-cyclodextrines.

(57) Described is a new process for the preparation of alkali clavulanate from an aqueous solution of crude clavulanic acid, which is obtained in a conventional manner after the fermentation with a clavulanic-acid-producing microorganism, extracted with an ethyl acetate solution of a hydrophobic  $\beta$ -CD derivative such as heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD in at least equimolar ratio with regard to clavulanic acid. The resulting novel inclusion complexes of clavulanic acid and hydrophobic  $\beta$ -CD derivative in a molar ratio about 1:1 are isolated, purified and then converted with an alkali base or alkali alkanoate to the desired alkali clavulanate, which is isolated. Furthermore, there are described new inclusion complexes of clavulanic acid and of its pharmaceutically acceptable alkali salts both with hydrophilic  $\beta$ -CD derivatives and with hydrophobic  $\beta$ -CD derivatives, processes for the preparation thereof and the use thereof for the preparation of galenic forms with immediate as well as with sustained action, in combination with amoxicillin trihydrate, which are valuable medicaments in the therapy of infectious diseases.

EP 0 578 231 A1

### Technical Field

The present invention belongs to the field of pharmaceutical industry and relates to novel inclusion complexes of clavulanic acid and of alkali salts thereof, such as potassium or sodium clavulanate, with hydrophilic or hydrophobic  $\beta$ -cyclodextrin (abbr.  $\beta$ -CD for  $\beta$ -cyclodextrin), to the preparation thereof and to the use thereof as intermediate compounds for the isolation of pure clavulanic acid or of alkali salts thereof as well as to the use thereof for the preparation of new stable galenic forms with immediate as well as sustained release.

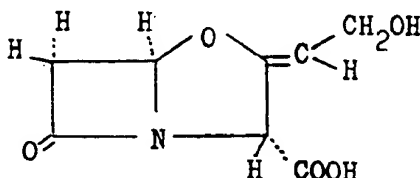
### Technical Problem

There exists a constant need for a new and simple process for the preparation of pure clavulanic acid and of pharmaceutically acceptable alkali salts thereof such as potassium clavulanate, whereby the desired compound with a high total yield and a high purity would be isolated from water solutions obtained in a usual manner after the fermentation by a microorganism producing clavulanic acid.

Furthermore, there existed a need for new stable galenic forms of clavulanic acid or of alkali salts thereof, such as potassium clavulanate, with  $\beta$ -lactam penicillinic or cephalosporinic antibiotics such as amoxicillin trihydrate or ticarcillin, wherein the clavulanic acid or alkali clavulanate would have an improved stability in an acidic medium existing in the gastric mucuous membrane, which would result in an improved relative biological applicability of the active component.

### Prior Art

Clavulanic acid is the generic name for (2R,5R,Z)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid, which is a known compound of the following formula:



Alkali salts and esters thereof are active as inhibitors of  $\beta$ -lactamases, i.e. they inhibit the action of  $\beta$ -lactamases produced by some gram-positive and gram-negative microorganisms.

In addition to being inhibitors of  $\beta$ -lactamases, clavulanic acid or its alkali salts also have a synergistic effect in combination with  $\beta$ -lactam antibiotics of penicillinic and cephalosporin class, therefore clavulanic acid or alkali salts thereof are used in galenic preparations to prevent a deactivation of  $\beta$ -lactam antibiotics.

In the literature, there is described the possibility of clavulanic acid production in a fermentative manner by means of various microorganisms of the strain *Streptomyces* such as *S. clavuligerus* NRRL 3585, *S. jumoninensis* NRRL 5741, *S. katsurahamanus* IFO 13716 and *Streptomyces* sp. P6621 FERM P 2804.

Clavulanic acid and its alkali salts were first described in GB 1,508,977. After the fermentation by means of the strain *S. clavuligerus* NRRL 3585, the process for the isolation of clavulanic acid from the fermentation broth, which is based on the use of anionic exchange resins and exacting purifications by chromatographic methods, is time-consuming.

In GB patent 1,543,563 a modified fermentative process using the strain *S. clavuligerus* NRRL 3685 is described, whereat the pH value of the medium is maintained in a range between 6.3 and 6.7, whereby the yield of desired compound is increased. Clavulanic acid salts such as potassium clavulanate are prepared by resalting from lithium clavulanate.

An improved process for the isolation and purification of clavulanic acid and of its alkali salts is described in EP patent 0026044. This process is based on the preparation of an intermediate salt of clavulanic acid with tert. butylamine, preferably in the form of its acetone solvate, which is prepared by treating the extract such as ethyl acetate extract, which was prepared according to the process in GB patent 1,508,977, containing impure clavulanic acid and which was previously discoloured by treating with activated charcoal, with tert. butylamine in an organic solvent, followed by the conversion of the acetone solvate of the salt of clavulanic acid with tert. butylamine, which was previously also additionally purified by

the recrystallization with alkali carbonates, bicarbonates, hydroxides or salts of alcanoic acid such as potassium 2-ethyl-hexanoate, to the pharmaceutically acceptable alkali salts of clavulanic acid such as potassium clavulanate.

##### 5 Technical Solution

This invention is based on the task to isolate clavulanic acid or pharmaceutically acceptable alkali salts thereof such as potassium clavulanate, from concentrated water solutions, which are obtained in the usual manner after the fermentation with a clavulanic-acid-producing microorganism, wherein clavulanic acid is present in a crude form, in a new, rapid and simple manner, whereby the desired compound would be obtained in an excellent total yield and with high purity. This aim was achieved so that a water solution of crude clavulanic acid in the form of alkali salts was acidified with sulfuric acid to a pH value between 1 and 3, whereupon the acidified water solution of crude clavulanic acid was immediately converted with a solution of a  $\beta$ -cyclodextrin hydrophobic derivative in an inert organic solvent such as ethyl acetate, the inclusion complex of clavulanic acid with the  $\beta$ -cyclodextrin hydrophobic derivative was isolated from organic phase, which complex was purified and reacted with alkali carbonate, bicarbonate, hydroxide or alkanoate such as potassium 2-ethyl-hexanoate in an inert organic solvent such as isopropanol or acetone to the desired alkali clavulanate, which was then isolated.

According to the invention, the water solution (purified concentrate after fermentation) contained from 10 to 20 mg/ml of clavulanic acid in the form of its alkali salt (determined by the HPLC).

The conversion of the acidified water solution of crude clavulanic acid with the solution of  $\beta$ -CD hydrophobic derivative was carried out in an inert organic solvent such as ethyl acetate. With regard to the concentration of clavulanic acid, which was previously determined by the HPLC, an equimolar amount or excess (up to 10%) of  $\beta$ -CD hydrophobic derivative was used. The volume of the used inert organic solvent was about three times greater than the volume of the water phase. The obtained inclusion complex was very stable in an acidic medium, which also affected the high yield of the conversion.

As the  $\beta$ -cyclodextrin hydrophobic derivatives there were used: heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD (also abbr. as triacetyl- $\beta$ -CD), in the nomenclature CAS (Chemical Abstracts Service) [23739-88-0] called  $\beta$ -cyclodextrin heneicosa acetate (the literature also reports abbr. TA- $\beta$ -CD), heptakis-(2,6-di-O-ethyl)- $\beta$ -CD (abbr. DE- $\beta$ -CD), heptakis-(2,3-di-O-ethyl)- $\beta$ -CD, heptakis-(2,3,6-tri-O-ethyl)- $\beta$ -CD (abbr. TE- $\beta$ -CD), O-carboxymethyl-O-ethyl- $\beta$ -CD, heptakis-2,6-di-O-pentyl- $\beta$ -CD, heptakis-2,3,6-tri-O-pentyl- $\beta$ -CD, heptakis-(3-O-acetyl-2,6-di-O-pentyl)- $\beta$ -CD, etc.

A short review of cyclodextrins is reported by O. Bekers et al. in Drug Development and Industrial Pharmacy, 17(11), 1503-1549 (1991), "Cyclodextrins in the pharmaceutical Field", wherein single cyclodextrin derivatives, terms or abbreviations, processes for the preparation of inclusion complexes, their characterization, the importance in pharmacy, etc. are described.

Hydrophobic  $\beta$ -CD are very poorly soluble in water. For example, the solubility in water of diethyl- $\beta$ -CD is  $5.0 \times 10^{-3}$  g/100 ml of water, and the solubility of triethyl- $\beta$ -CD is  $1.8 \times 10^{-3}$  g/100 ml of water.

Clavulanic acid itself or alkali salts thereof are hydrophilic compounds that are very well soluble in water. Inclusion complexes of clavulanic acid with  $\beta$ -CD hydrophobic derivatives such as the inclusion complex of clavulanic acid with heptakis-2,3,6-tri-O-acetyl- $\beta$ -CD, which are formed in this step, are novel compounds, not yet described in the literature.

The obtained inclusion complexes are then isolated and, if desired, also purified, most conveniently by washing with water or by the recrystallisation. The yield in this step is very high and amounts to over 90%. In comparison with the process for the isolation of clavulanic acid from ethyl acetate extract prepared in known manners and containing crude clavulanic acid, which is carried out via intermediate salts of clavulanic acid with tert. butylamine as described in EP 0026044, the process of the invention is advantageous because clavulanic acid bound in an inclusion complex such as the complex with triacetyl- $\beta$ -CD, is significantly more stable at isolation conditions (low pH) than in the noncomplexed form (the complexation is selective for clavulanic acid). The separation of the water phase from the organic phase containing TA- $\beta$ -CD is made easier. Due to its stability, the inclusion complex can be saved for a longer period of time (some weeks), if necessary. Since the complex is crystalline and water-insoluble, it can be purified simply by washing with water to eliminate the water-soluble accompanying substances.

The intermediate inclusion complex of clavulanic acid with hydrophobic derivative of  $\beta$ -CD such as triacetyl- $\beta$ -CD, is then reacted with alkali carbonate, bicarbonate, hydroxide or alkanoate such as potassium 2-ethyl-hexanoate, in an inert organic solvent such as isopropanol, whereby the desired alkali clavulanate is separated and then isolated.

All steps of the conversion are carried out at about ambient temperature.

The last step of conversion is also carried out easily and rapidly, whereat in a solution of an inert organic solvent such as isopropanol or acetone, the inclusion complex of clavulanic acid with triacetyl- $\beta$ -CD is decomposed by the addition of alkali 2-ethyl-hexanoate in the same solvent, and the alkali clavulanate such as potassium clavulanate is separated.

5 Though in the step of the preparation of the intermediate complex there are required greater amounts of triacetyl- $\beta$ -CD, which is added in at least equimolar ratio with regard to clavulanic acid (the molecular weight of a triacetyl- $\beta$ -CD is about 10 times greater than the molecular weight of clavulanic acid), the used  $\beta$ -CD hydrophobic derivative can, after the isolation of the alkali clavulanate from the organic solvent solution, easily be recycled and returned back to the process.

10 A variant process for the isolation of potassium clavulanate from concentrated water solutions after the fermentation with a clavulanic-acid-producing microorganism is based on the conversion of the inclusion complex of clavulanic acid with triacetyl- $\beta$ -CD, prepared in the above mentioned manner, with N,N'-diisopropylethylenediamine, whereat the obtained N,N'-diisopropylethylenediammonium diclavulanate is converted to the desired compound in the manner described in our Slovenian patent application SI P-92 00

15 382. This variant process is applied if a very high purity of the desired compound is required or in the cases when after fermentation the concentrated water solutions of clavulanic acid are not sufficiently pure.

The use of  $\beta$ -CD hydrophobic derivatives for the isolation of therapeutically effective active component such as clavulanic acid or alkali salts thereof is a new and non-obvious process not yet described in the literature and, in addition, these complexes are useful for the preparation of galenic forms with controlled

20 release of the active component since the inclusion complex is retardative by itself. A further object of the invention is the preparation of inclusion complexes of alkali clavulanate with  $\beta$ -CD hydrophilic derivatives and their use in the preparation of stable galenic forms for oral application such as capsules or tablets (effervescent, dispersive, chewing etc.) containing, in addition to the pharmaceutically

25 acceptable carrier, a therapeutically effective amount of amoxicillin trihydrate and alkali salt of clavulanic acid, whereat the weight ratio between amoxicillin trihydrate and equivalent of alkali clavulanate bound in the complex amounts from 10:1 to 1:1. From GB patent 2,005,538, there are known solid galenic forms containing, in addition to the pharmaceutically acceptable carrier, amoxicillin trihydrate and potassium clavulanate, whereat the preferred

30 ratio of amoxicillin trihydrate and potassium clavulanate is 2:1. The recommended dose (in paediatrics) contains 250 mg of amoxicillin (in trihydrate form) and 125 mg of clavulanic acid (in potassium salt form). The recommended ratio of the components for adult patients is 4:1. Solid galenic forms such as capsules and tablets, which are described in this patent, have an improved stability. They are prepared in conditions of low relative humidity (less than 30%) and the package also contains a drying agent such as silicagel

35 because of the high hygroscopicity of clavulanic acid or alkali salts thereof. The inclusion complexes of clavulanic acid or of alkali salts thereof, such as potassium clavulanate, with  $\beta$ -CD hydrophilic derivatives of the invention may be prepared by known processes which are described in the literature, e.g. by M. Kurozumi et al., Chem. Pharm. Bull. 23(12), 3062-3068 (1975) or Acta Pharm. Technol. 36(1), 1-6 (1990), i.e. by complexing of active component with selected cyclodextrin, or they may

40 be prepared in a novel manner, not yet described in the literature, which is a further object of the present invention. According to the invention, the inclusion complex of clavulanic acid is reacted with hydrophobic  $\beta$ -CD such as triacetyl- $\beta$ -CD, which is obtained in the above-mentioned manner, in a water solution of an alkali base such as alkali carbonate, bicarbonate, hydroxide or alkanoate such as potassium 2-ethylhexanoate,

45 directly with  $\beta$ -CD hydrophilic derivative, whereafter the desired compound is isolated and purified if desired. This process representing substantially a recomplexing of one inclusion complex to another is, with regard to the aim of the invention, shorter than the above-mentioned one since no previous preparation of alkali clavulanate is necessary.

As  $\beta$ -CD hydrophilic derivatives there may be used all known compounds of this kind such as heptakis-

50 (2,6-di-O-methyl)- $\beta$ -CD, monomethyl- $\beta$ -CD (Me- $\beta$ -CD), heptakis-(2,3,6-tri-O-methyl)- $\beta$ -CD, hydroxypropyl- $\beta$ -CD, hydroxyethyl- $\beta$ -CD, dihydroxypropyl- $\beta$ -CD,  $\beta$ -CD branched derivatives such as glucosyl- $\beta$ -CD, maltosyl- $\beta$ -CD, dimaltosyl- $\beta$ -CD, diglucosyl- $\beta$ -CD and other water soluble or hydrophilic derivatives of  $\beta$ -CD.

In a dose unit, galenic forms of the invention may contain, in addition to the pharmaceutically acceptable carrier and other adjuvants, the same amounts of amoxicillin trihydrate and of equivalent of alkali

55 clavulanate such as potassium clavulanate in a complex as commercial preparations.

It was unexpectedly found that by complexing clavulanic acid or its alkali salt, the stability of clavulanic acid at pH 1.2 and the temperature of 37 °C, i.e. in the acidic medium as it is present in gastric juice, is substantially improved, whereby the repeating in the extent of the absorption of clavulanic acid from the

gastrointestinal tract or the biological availability of the active component is improved.

The stability of clavulanic acid itself in conditions being present in gastric juice is exceptionally low (the time of 95% decomposition of clavulanic acid at 37 °C is 26 minutes), whereas, due to the complexing of the clavulanic acid with  $\beta$ -CD, its acid stability is improved, which makes possible the absorption of clavulanic acid from the gastrointestinal tract to the blood in a greater extent, which is much less dependent on the pH of the medium (as in the gastric mucuous membrane). This further contributes to a greater therapeutical effectiveness in a combination with amoxicillin trihydrate since the inhibition of  $\beta$ -lactamases is increased. This certainly means that in order to achieve the same therapeutical effect as by a commercial preparation (Augmentin), lower doses of clavulanic acid or of its alkali salt such as potassium clavulanate may be used.

A further object of the invention are inclusion complexes of clavulanic acid or of its alkali salts with  $\beta$ -CD hydrophobic derivatives such as heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD, which may be obtained by the above-mentioned process or may be prepared from both components in known manners which are described in the literature, which represent a sustained release system, wherefrom the active component is released in a controlled manner as shown in the graph. The inclusion complex itself represents a sustained release complex and therefore no other sustained release components in the galenic form are necessary. To galenic forms with a sustained release of the active component, in addition to the inclusion complex and amoxicillin trihydrate, adjuvants such as desintegrators, fillers, colouring agents, sweetening agents etc. may be added if necessary.

A further object of the invention is to prepare stable galenic forms with a controlled release of the active component, which contain amoxicillin trihydrate and a mixture of two inclusion complexes of clavulanic acid or of its alkali salts, whereat one of the  $\beta$ -cyclodextrin derivatives used for complexing is hydrophilic and the other is hydrophobic.

In this case the release of active component from the galenic form may be controlled in the desired extent by a suitably adapted ratio of the two types of inclusion complexes.

Owing to the high stability in the acidic medium of gastric juice and a suitable sustained rapidity of releasing clavulanic acid from the system, these complexes have an improved biological applicability.

All inclusion complexes of clavulanic acid and of its alkali salts with hydrophilic or hydrophobic  $\beta$ -CD derivatives according to the invention are formed in a molar ratio of about 1:1.

From US patent 4,869,904, a sustained release system of inclusion complexes of the active components with  $\beta$ -CD hydrophobic alkylated derivatives such as heptakis-(2,3-di-O-ethyl)- $\beta$ -CD is known.

According to the invention, in addition to the heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD also O-carboxymethyl-O-ethyl- $\beta$ -CD, heptakis-2,6-di-O-pentyl- $\beta$ -CD, heptakis-2,3,6-tri-O-pentyl- $\beta$ -CD or heptakis-(3-O-acetyl-2,6-di-O-pentyl)- $\beta$ -CD may be used as the hydrophobic  $\beta$ -CD derivative.

No toxic effect was observed in any of the performed tests. Thus it may be considered that the toxicity of the inclusion complexes is as low as the toxicity of clavulanic acid or its salts.

The invention is illustrated but in no way limited by the following Examples.

#### Example 1

Preparation of the inclusion complex of potassium clavulanate with methyl- $\beta$ -cyclodextrin (Me- $\beta$ -CD)

Me- $\beta$ -CD (13.10 g, 10 mmol) was dissolved in water for injections (25 ml) under stirring at ambient temperature and then to the solution potassium clavulanate (2.377 g, 10 mmol) was slowly added under constant stirring. The obtained solution was stirred at ambient temperature in the dark for 10 minutes, then it was filtered, the filtrate was frozen in a stream of liquid nitrogen and then it was freeze-dried.

There was obtained a slightly yellowish microcrystalline title complex (15.25 g, 98.5%). The complex contained 15.2% of potassium clavulanate as it was determined by the HPLC method.

Figs. 1 and 2 illustrate <sup>1</sup>NMR and DSC (differential scanning calorimetry) spectra of the title complex.

#### Example 2

Preparation of the inclusion complex of potassium clavulanate with 2-hydroxypropyl- $\beta$ -CD (2-HP- $\beta$ -CD)

It was proceeded in the same way as in Example 1, only that methyl- $\beta$ -cyclodextrin was replaced by 2-hydroxypropyl- $\beta$ -cyclodextrin (13.80 g, 10 mmol).

There was obtained a microcrystalline title complex (16.0 g, 99.3%). The complex contained 14.6% of potassium clavulanate as it was determined by the HPLC method.

Figs. 3 and 4 illustrate <sup>1</sup>NMR and DSC spectra of the title complex.

#### Example 3

- 5 Preparation of the inclusion complex of potassium clavulanate with 2-hydroxyethyl- $\beta$ -cyclodextrin (2-HE- $\beta$ -CD)

It was proceeded in the same way as in Example 1, only that methyl- $\beta$ -cyclodextrin was replaced by 2-hydroxyethyl- $\beta$ -CD (14.40 g, 10 mmol).

- 10 There was obtained microcrystalline title complex (16.2 g, 96.7%). The complex contained 13.9% of potassium clavulanate as it was determined by the HPLC method.

Figs. 5 and 6 illustrate <sup>1</sup>NMR and DSC spectra of the title complex.

#### Example 4

15

Process for the isolation of clavulanic acid or of potassium salt thereof

- A water solution (1.25 l) containing clavulanic acid (20 g, 0.1 mol) was acidified with conc. sulfuric acid (15 ml) to the pH value of 1.3 and then the acidified water solution was immediately extracted in a centrifuge with ethyl acetate (4.0 l) in which heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD (201.78 g, 0.1 mol) was dissolved. The phases were separated and the organic phase was washed with water (250 ml) on a separative column. Subsequently, the solvent was evaporated from the organic phase and the residue was dried in vacuum at the temperature of 35 °C to a constant weight. There was obtained a crude inclusion complex (212.9 g, 96%) of clavulanic acid with heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD, which was then digested  
25 in water (500 ml) under vigorous stirring. The complex was then filtered off, washed with water (3 x 50 ml) and dried in vacuum at a temperature up to 35 °C to the constant weight (210.4 g). The complex contained 8.1% of clavulanic acid as it was determined by the HPLC method.

- The purified inclusion complex (210.4 g) of clavulanic acid with triacetyl- $\beta$ -CD was dissolved in acetone (250 ml), the solid particles were filtered off and a solution (100 ml, 20%) of potassium 2-ethyl-hexanoate  
30 in isopropanol was slowly added dropwise under stirring. Potassium clavulanate separated and it was filtered off, washed with isopropanol (3 x 15 ml) and dried in vacuum at a temperature up to 35 °C to the constant weight. The yield of potassium clavulanate was 18.0 g (88 %) (the clavulanic acid content of 83.0 % was determined by the HPLC).

- Figs. 7 and 8 illustrate NMR and DSC spectra of the inclusion complex of clavulanic acid with triacetyl- $\beta$ -CD.  
35

#### Example 5

- Preparation of the inclusion complex of potassium clavulanate with methyl- $\beta$ -cyclodextrin (Me- $\beta$ -CD) by  
40 recomplexing from the inclusion complex of clavulanic acid with triacetyl- $\beta$ -CD

- The inclusion complex (100 g) of clavulanic acid with heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD, which was prepared in the same way as in Example 4, was suspended in a 3% aqueous potassium carbonate solution (200 ml), in which methyl- $\beta$ -CD (53.6 g, 40.9 mmol) was dissolved. The suspension was stirred for 5 min at  
45 ambient temperature, the solid particles were filtered off and the filtrate was frozen in liquid nitrogen and then freeze-dried. There was obtained a yellowish microcrystalline inclusion complex (56.7 g, 91.9%) of potassium clavulanate with methyl- $\beta$ -CD. The complex contained 14.9% of potassium clavulanate as it was determined by the HPLC method.

- In the same manner inclusion complexes of other alkali clavulanates can be prepared.  
50

#### Example 6

- Stability test of complexed and uncomplexed potassium clavulanate in artificial gastric juice (pH 1.2) at the temperature of 37 °C  
55

For the test the following samples were used:

- 1) potassium clavulanate (abbr. KK)
- 2) inclusion complex of KK with methyl- $\beta$ -CD (abbr. KK-Me- $\beta$ -CD)

3) inclusion complex of KK with 2-hydroxypropyl- $\beta$ -CD (abbr. KK-HP- $\beta$ -CD)

4) inclusion complex of clavulanic acid with triacetyl- $\beta$ -CD (abbr. KA-TA- $\beta$ -CD).

Medium: artificial gastric juice - aq.HCl/NaCl buffer, pH 1.2, according to USP XXII.

Concentration of clavulanic acid (abbr. KA) in samples: 4 mg/ml.

5 The samples 1 to 3 were dissolved under stirring in an artificial gastric juice (100 ml) thermostated at 37 °C, at the concentration of 4 mg/ml with regard to the clavulanic acid. Subsequently, 100  $\mu$ l aliquots were taken at 5 min intervals for analysis by the HPLC, until a more than 95% KA decomposition was found. The solution was stirred all the time at the temperature of 37 °C  $\pm$  0.5 °C.

10 Separately, a series of ten 10 ml aliquots of KA-TA- $\beta$ -CD suspension in a buffer of a pH of 1.2 (conc. of KA = 4 mg/ml), which were constantly stirred during thermostating at 37 °C, was prepared. At time intervals of 5 to 10 min, the individual aliquots were filtered (membrane filter 0.4  $\mu$ m) and in the clear filtrate the concentration of clavulanic acid was determined by HPLC. The KA content at times up to 90 min of thermostating was noted.

15 The results of measurements after 15, 30, 45, 60 and 90 min of thermostating the samples at 37 °C in a buffer at pH 1.2 are shown in following Table.

| Thermostating time [min] | KA (undecomp.) as to the initial KA conc.(100%) in samples [%] |                    |                    | Released and undecomp. KA in supernatant above suspension [%] |
|--------------------------|--|--------------------|--------------------|---|
|                          | KK   | KK-Me- $\beta$ -CD | KK-HP- $\beta$ -CD | KA-TA- $\beta$ -CD  |
| 15 min                   | 18.1   | 26.6               | 24.7               | 9.6   |
| 30 min                   | 1.3  | 11.2               | 10.2               | 10.9  |
| 45 min                   | 0  | 4.1                | 3.4                | 10.5  |
| 60min                    |  |                    |                    | 11.0  |
| 90min                    |  |                    |                    | 10.6  |

30 In Diagram 1, the times of 95% decomposition of clavulanic acid ( $t_{95\%}$ ) are shown:

|              |                    |          |
|--------------|--------------------|----------|
| $t_{95\%}$ : | KK                 | 26 min   |
|              | KK-Me- $\beta$ -CD | 42 min   |
|              | KK-HP- $\beta$ -CD | 40 min   |
|              | KA-TA- $\beta$ -CD | > 90 min |

40 From the obtained results it is evident that the complexing of potassium clavulanate with hydrophilic Me- $\beta$ -CD and HP- $\beta$ -CD substantially increases the stability of potassium clavulanate and clavulanic acid in artificial gastric juice (pH 1.2, 37 °C). The ratio in undecomposed clavulanic acid after 30 min. is about 1:8 in the favour of complexed potassium clavulanate (see Diagram 1).

45 It is further evident from Diagram 1 that within 90 minutes the KA concentration in the supernatant above suspension of hydrophobic inclusion complex KA-TA- $\beta$ -CD keeps constant (it contains 10 to 11% of the starting KA), which represents a sustained and constant release of KA from the complex. The decomposed clavulanic acid is simultaneously replaced by a new amount of clavulanic acid from the complex KA-TA- $\beta$ -CD as a result of the concentration equilibrium of complexed and uncomplexed clavulanic acid. Such a profile of the release of clavulanic acid points to the applicability of the complexes of clavulanic acid with hydrophobic  $\beta$ -CD derivatives for sustained release forms.

50 After 10 minutes, a 90 ml aliquot of KA-TA- $\beta$ -CD suspension was filtered and the precipitate was washed out with 10 ml of fresh buffer according to the USP XXII. Subsequently, the precipitate was quantitatively transferred to a 10 ml measuring flask, dissolved in a mixture of MeOH:H<sub>2</sub>O = 1:1 and filled with the solvent up to the mark. By the HPLC analysis it was determined that the sample contained 0.78 mg of KA/ml, which is 19% of the starting KA amount in the complex. These results show that from the complex KA-TA- $\beta$ -CD the KA is released constantly and in a sustained manner.

55 The complexed part of clavulanic acid remained stable in acidic medium and the slow release at simultaneous absorption from the stomach to blood made possible a substantially greater extent of absorption than other hitherto known forms.

There was also determined the release of clavulanic acid or its potassium salt from the complex with hydrophobic triacetyl- $\beta$ -CD in artificial intestinal juice (pH 7.2, buffer according to the USP XXII), which also ran constantly and about twice faster than in artificial gastric juice with pH 1.2.

From the above discussion it may be concluded that the inclusion complex of clavulanic acid with triacetyl- $\beta$ -CD is a suitable sustained release system for peroral forms.

#### Example 7

##### X-ray powder diffraction

In the Table below there are shown the lattice distances  $d(\text{nm})$  and intensity ( $I$ ) of X-ray powder diffraction patterns of potassium clavulanate (abbr. KK), methyl- $\beta$ -CD (abbr. Me- $\beta$ -CD), 2-hydroxypropyl- $\beta$ -CD (HP- $\beta$ -CD), 2-hydroxyethyl- $\beta$ -CD (abbr. HE- $\beta$ -CD) and of the inclusion complexes of potassium clavulanate with the enumerated  $\beta$ -CD derivatives (abbr. KK-Me- $\beta$ -CD, KK-HP- $\beta$ -CD and KK-HE- $\beta$ -CD), which were taken by Philips PW 1710 diffractometer on an Al-substrate and at  $\lambda = 0.15418 \text{ nm (CuK}\alpha\text{)}$ .

| Sample             | $d(\text{nm})$ | $I$  |
|--------------------|----------------|------|
| KK                 | 0.35389        | 1694 |
|                    | 0.29359        | 1426 |
|                    | 0.37586        | 1176 |
| Me- $\beta$ -CD    | 0.49695        | 1454 |
|                    | 0.46853        | 1422 |
|                    | 0.49081        | 1404 |
| KK-Me- $\beta$ -CD | 0.46426        | 1100 |
|                    | 0.49217        | 1072 |
|                    | 0.47133        | 1068 |
| HP- $\beta$ -CD    | 0.48196        | 1576 |
|                    | 0.46370        | 1538 |
|                    | 0.48850        | 1532 |
| KK-HP- $\beta$ -CD | 0.46018        | 1198 |
|                    | 0.45376        | 1164 |
|                    | 0.47544        | 1140 |
| HE- $\beta$ -CD    | 0.47459        | 1432 |
|                    | 0.46811        | 1360 |
|                    | 0.35968        | 1298 |
| KK-HE- $\beta$ -CD | 0.45327        | 1046 |
|                    | 0.44711        | 1018 |
|                    | 0.47514        | 1018 |

#### Example 8

Process for the isolation of clavulanic acid or of potassium salt thereof

To an ethylacetate solution (1 l) of the inclusion complex of clavulanic acid with heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD (the concentration of clavulanic acid was 10 mg/ml as determined by the HPLC method), which was obtained according to the process of Example 4, N,N'-diisopropylethylenediamine (5 ml) was slowly added dropwise under vigorous stirring within 10 min. The resulting suspension was stirred for another 30 min, the produced precipitate was filtered off and then dissolved in 10 ml of water. Acetone (200 ml) was added to the solution, whereat N,N'-diisopropyl-ethylenediammonium diclavulanate (6.1 g) was separated in the form of fine crystals, which were filtered off and redissolved in water (5 ml). Isopropanol (95 ml) was added to the solution and then a solution of potassium 2-ethylhexanoate (10 ml, 2M) in isopropanol was slowly added dropwise under vigorous stirring within 15 min, whereat potassium clavulanate was separated, which was filtered off, washed with isopropanol (3 x 10 ml) and dried in vacuum.



at a temperature up to 35 °C to constant weight. Thus there was obtained potassium clavulanate (2.64 g, 77%) (USP grade, clavulanic acid content 82.8% as determined by HPLC method).

#### Example 9

5

Preparation of inclusion complex of potassium clavulanate with triacetyl- $\beta$ -cyclodextrin

Potassium clavulanate (237.3 mg, 1 mmol) was dissolved in water for injections (10 ml) under stirring at ambient temperature and then a solution of triacetyl- $\beta$ -cyclodextrin (TA- $\beta$ -CD; 2.02 g, 1 mmol) in  
10 isopropanol (90 ml) was added. The reaction mixture was stirred for 5 min at ambient temperature, subsequently the solvents was evaporated at reduced pressure, at first on a rotary evaporator at the temperature of 30 °C and then the residue was frozen and freeze-dried. Microcrystalline title complex (2.25 g, 99%) was obtained. The complex contained 9.1% of potassium clavulanate (corresponding to 0.9 mol of potassium clavulanate/1 mol of triacetyl- $\beta$ -CD) as determined by the HPLC method.

16 The DSC analysis corresponded to the title complex.

The NMR spectrum corresponded to potassium clavulanate and triacetyl- $\beta$ -CD.

#### Example 10

20 Dispersion tablets containing clavulanic acid (125 mg) and amoxicillin (500 mg)

|    | Ingredients   | mg/tablet | %         |
|----|---|-----------|-----------|
| 25 | inclusion complex of potassium clavulanate with Me- $\beta$ -CD           | 833.3     | 27.78     |
|    | amoxicillin trihydrate (corresponding to 500 mg of anhydrous amoxicillin) | 574.0     | 19.13     |
|    | crospovidone (Kollidon CL)  | 50.0      | 1.67      |
|    | saccharin   | 10.0      | 0.33      |
|    | flavourings (fruit)   | 35.0      | 1.17      |
| 30 | magnesium stearate  | 21.0      | 0.70      |
|    | colloidal silica (Aerosil 200)  | 10.0      | 0.33      |
|    | microcrystalline cellulose (Avicel pH 101)                                | ad 3000.0 | ad 100.00 |

35 The equivalents of amoxicillin and clavulanic acid were mixed homogeneously and then briquetted with a part of the filler (microcrystalline cellulose). The briquettes were crushed and sieved through an oscillation sieve with a mesh size of 1.5 mm. The obtained granulate was mixed with the remaining adjuvants and tableted on a rotary tableting machine.

The tablets rapidly disintegrate in water giving a fine dispersion suitable for therapeutic application.

40

#### Example 11

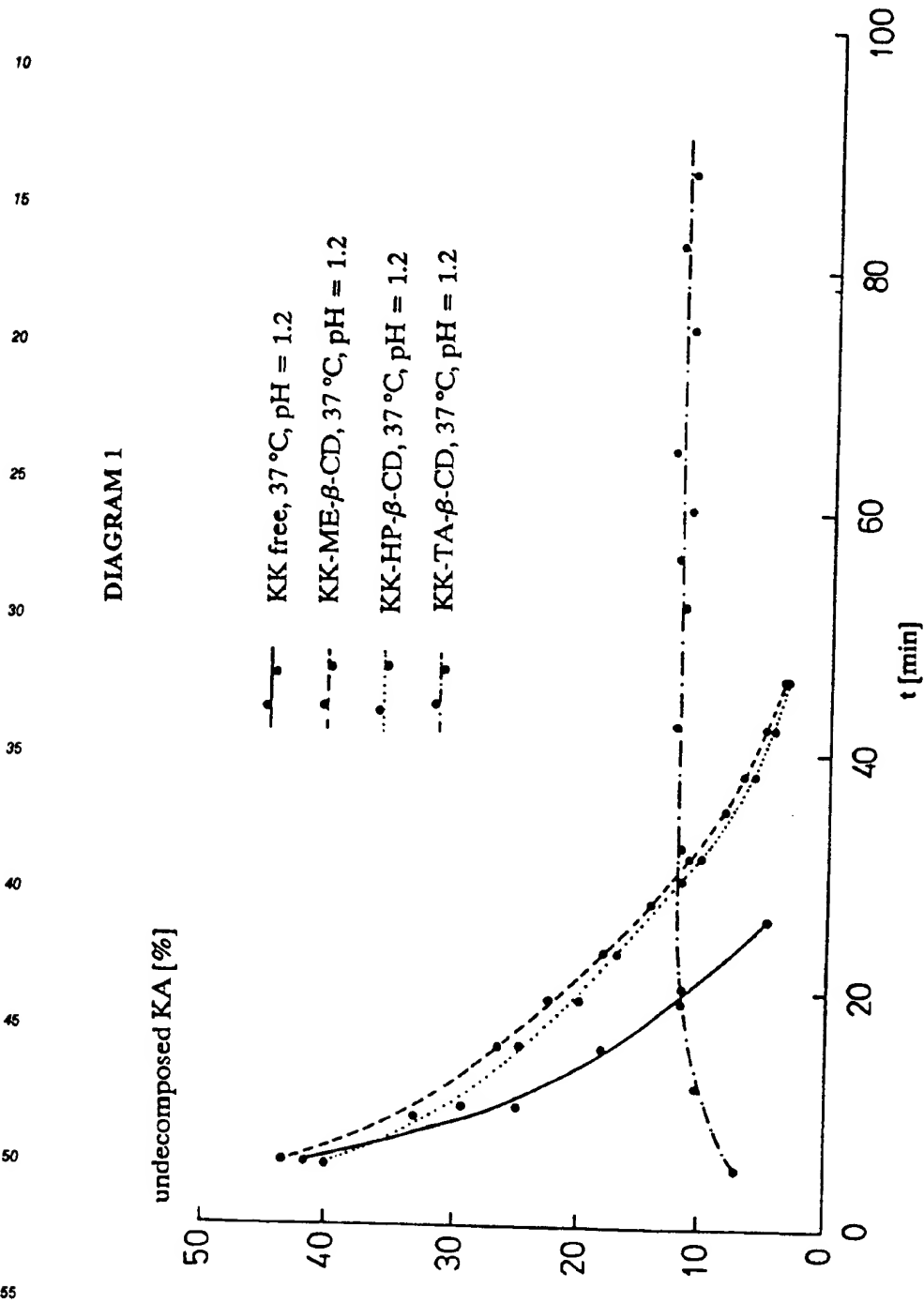
Chewing tablets containing clavulanic acid (125 mg) and amoxicillin (500 mg)

45

|    | Ingredients   | mg/tablet | %         |
|----|---|-----------|-----------|
|    | inclusion complex of potassium clavulanate with Me- $\beta$ -CD           | 833.3     | 27.78     |
|    | amoxicillin trihydrate (corresponding to 500 mg of anhydrous amoxicillin) | 574.0     | 19.13     |
|    | crospovidone (Kollidon CL)  | 5.0       | 0.17      |
| 50 | polyvinyl pyrrolidone   | 60.0      | 2.00      |
|    | colloidal silica (Aerosil 200)  | 10.0      | 0.33      |
|    | saccharin   | 10.0      | 0.33      |
|    | flavourings (fruit)   | 35.0      | 1.17      |
| 55 | magnesium stearate  | 15.0      | 0.50      |
|    | microcrystalline cellulose (Avicel pH 101)                                | 60.0      | 2.00      |
|    | mannitol  | ad 3000.0 | ad 100.00 |

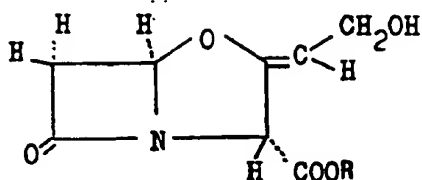
The equivalents of amoxicillin and clavulanic acid were homogeneously mixed together with a part of the fillers (microcrystalline cellulose and mannitol) and the obtained mixture of powders was briquetted. The briquettes were crushed and sieved through an oscillation sieve with a mesh size of 1.5 mm. The obtained granulate was mixed with the remaining adjuvants and tableted.

5 Tablets were suitable for chewing.



## Claims

1. Inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof of the formula I:



I

wherein R represents hydrogen or an alkali metal, with hydrophilic and hydrophobic  $\beta$ -cyclodextrin ( $\beta$ -CD) derivatives.

2. Inclusion complexes according to claim 1, characterized in that the molar ratio of clavulanic acid or of pharmaceutically acceptable alkali salts thereof and of hydrophilic or hydrophobic  $\beta$ -cyclodextrin derivatives amounts to about 1:1.
3. Inclusion complexes according to claim 1, characterized in that the hydrophilic  $\beta$ -cyclodextrin derivatives are selected from the group comprising heptakis-(2,6-di-O-methyl)- $\beta$ -cyclodextrin, heptakis-(2,3,6-tri-O-methyl)- $\beta$ -cyclodextrin, monomethyl- $\beta$ -cyclodextrin, 2-hydroxypropyl- $\beta$ -CD, 3-hydroxypropyl- $\beta$ -CD, 2-hydroxyethyl- $\beta$ -CD, 2,3-dihydroxypropyl- $\beta$ -CD, 6-O- $\alpha$ -D-glucosyl- $\beta$ -CD, 6-O- $\alpha$ -D-maltosyl- $\beta$ -CD, diglucosyl- $\beta$ -CD, dimaltosyl- $\beta$ -CD, maltotriosyl- $\beta$ -CD, carboxymethyl- $\beta$ -CD, carboxyethyl- $\beta$ -CD.
4. Inclusion complexes according to claim 1, characterized in that the hydrophobic  $\beta$ -cyclodextrin derivatives are selected from the group comprising heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD, heptakis-(2,6-di-O-ethyl)- $\beta$ -CD, heptakis-(2,3-di-O-ethyl)- $\beta$ -CD and heptakis-(2,3,6-tri-O-ethyl)- $\beta$ -CD, O-carboxymethyl-O-ethyl- $\beta$ -CD, heptakis-2,6-di-O-pentyl- $\beta$ -CD, heptakis-2,3,6-tri-O-pentyl- $\beta$ -CD, heptakis-(3-O-acetyl-2,6-di-O-pentyl)- $\beta$ -CD.
5. A process for the preparation of inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof of formula I of claim 1 with hydrophobic  $\beta$ -CD derivatives, characterized in that a solution of clavulanic acid or of pharmaceutically acceptable alkali salts thereof in an inert organic solvent is reacted with, hydrophobic  $\beta$ -CD derivatives at a temperature from 10 to 30 °C, and the desired compound is isolated and purified.
6. A process for the preparation of inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof of formula I of claim 1 with hydrophilic  $\beta$ -CD derivatives, characterized in that a solution of clavulanic acid or of pharmaceutically acceptable alkali salts thereof in water or in lower aliphatic alcohols or in their aqueous solutions is reacted with hydrophilic  $\beta$ -CD derivatives at a temperature from 10 to 30°C and the desired compound is isolated.
7. A process for the preparation of inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof with hydrophilic  $\beta$ -CD derivatives, characterized in that the inclusion complex of clavulanic acid with a hydrophobic  $\beta$ -CD derivative is reacted with a hydrophilic  $\beta$ -CD derivative in an aqueous solution of an alkali carbonate, bicarbonate, hydroxide or alkanoate at a temperature from 10 to 30 °C and the desired compound is isolated.
8. The process according to claim 7, characterized in that in the starting inclusion complex of clavulanic acid with a hydrophobic  $\beta$ -CD derivative, heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD is used as the hydrophobic component.
9. A process for the preparation of an alkali clavulanate, characterized in that the concentrate of a clear aqueous solution of crude clavulanic acid in the form of an alkali salt is acidified with sulfuric acid to a

pH value between 1 and 3 and subsequently the acidified aqueous solution of crude clavulanic acid is immediately reacted with the solution of a hydrophobic  $\beta$ -CD derivative in an inert organic solvent, the inclusion complex of clavulanic acid with hydrophobic  $\beta$ -CD derivative is isolated from the organic phase, purified and, with an alkali carbonate, bicarbonate, hydroxyde or alkanolate in an inert organic solvent, converted to the desired compound, which is then isolated.

10. The process for the preparation of an alkali clavulanate according to claim 9, characterized in that the hydrophobic  $\beta$ -CD derivative is selected from the group comprising heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD, heptakis-(2,6-di-O-ethyl)- $\beta$ -CD, heptakis-(2,3-di-O-ethyl)- $\beta$ -CD and heptakis-(2,3,6-tri-O-ethyl)- $\beta$ -CD.
11. The process for the preparation of an alkali clavulanate according to claim 9, characterized in that alkali 2-ethyl hexanoate is used as the alkali alkanolate.
12. The use of inclusion complexes of clavulanic acid with hydrophobic  $\beta$ -CD derivatives according to claims 9 and 10 as intermediate compounds for the preparation of alkali clavulanate.
13. Use of the inclusion complex of clavulanic acid with heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD as intermediate compound for the preparation of alkali clavulanate.
14. Use of inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof with hydrophilic  $\beta$ -CD derivatives for the preparation of stable galenic forms for oral application, characterized in that the galenic forms contain a therapeutically effective amount of amoxicillin trihydrate and of the inclusion complex of clavulanic acid and of its alkali salts with a hydrophilic  $\beta$ -CD derivative and a pharmaceutically acceptable carrier, wherein the ratio of amoxicillin trihydrate and of clavulanic acid and its alkali salts is from 10:1 to 1:1.
15. The use of inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof with hydrophilic  $\beta$ -CD derivatives according to claim 14, characterized in that the hydrophilic  $\beta$ -CD derivatives are selected from the group comprising heptakis-(2,6-di-O-methyl)- $\beta$ -CD, heptakis-(2,3,6-tri-O-methyl)- $\beta$ -CD, monomethyl- $\beta$ -CD, 2-hydroxypropyl- $\beta$ -CD, 2-hydroxyethyl- $\beta$ -CD, glucosyl- $\beta$ -CD, maltosyl- $\beta$ -CD, dimaltosyl- $\beta$ -CD and diglucosyl- $\beta$ -CD.
16. The use of inclusion complexes of clavulanic acid and of pharmaceutically acceptable alkali salts thereof with hydrophilic  $\beta$ -CD derivatives according to claims 14 and 15, characterized in that the galenic forms are tablets, capsules, granules, syrups, suspensions, intramammary suspensions and optionally also injections.
17. Stable galenic forms with sustained release of the active component, characterized in that they contain a therapeutically active amount of amoxicillin trihydrate and of the inclusion complex of clavulanic acid or of its alkali salts with a hydrophobic  $\beta$ -CD derivative selected of the group comprising heptakis-(2,3,6-tri-O-acetyl)- $\beta$ -CD, O-carboxymethyl-O-ethyl- $\beta$ -CD, heptakis-2,6-di-O-pentyl- $\beta$ -CD, heptakis-2,3,6-tri-O-pentyl- $\beta$ -CD and heptakis-(3-O-acetyl-2,6-di-O-pentyl)- $\beta$ -CD and other adjuvants, wherein the ratio of amoxicillin trihydrate and of clavulanic acid or of its pharmaceutically acceptable alkali salts is from 10:1 to 1:1.
18. Stable galenic forms with sustained release of the active component according to claim 17, characterized in that they are formed in a form suitable for oral application such as a tablet or capsule or optionally in the form of an oil intramuscular injection.
19. Stable galenic forms with sustained release of the active component, characterized in that they contain a therapeutically active amount of amoxicillin trihydrate and of a mixture of the inclusion complex of clavulanic acid or of its alkali salts with a hydrophobic  $\beta$ -CD derivative and a hydrophilic  $\beta$ -CD derivative and other adjuvants, wherein the ratio of amoxicillin trihydrate and of clavulanic acid or its pharmaceutically acceptable alkali salts is from 10:1 to 1:1.
20. A method for treating bacterial infections in humans and animals, characterised in that the stable galenic forms according to any one of claims 14 to 19 are used.

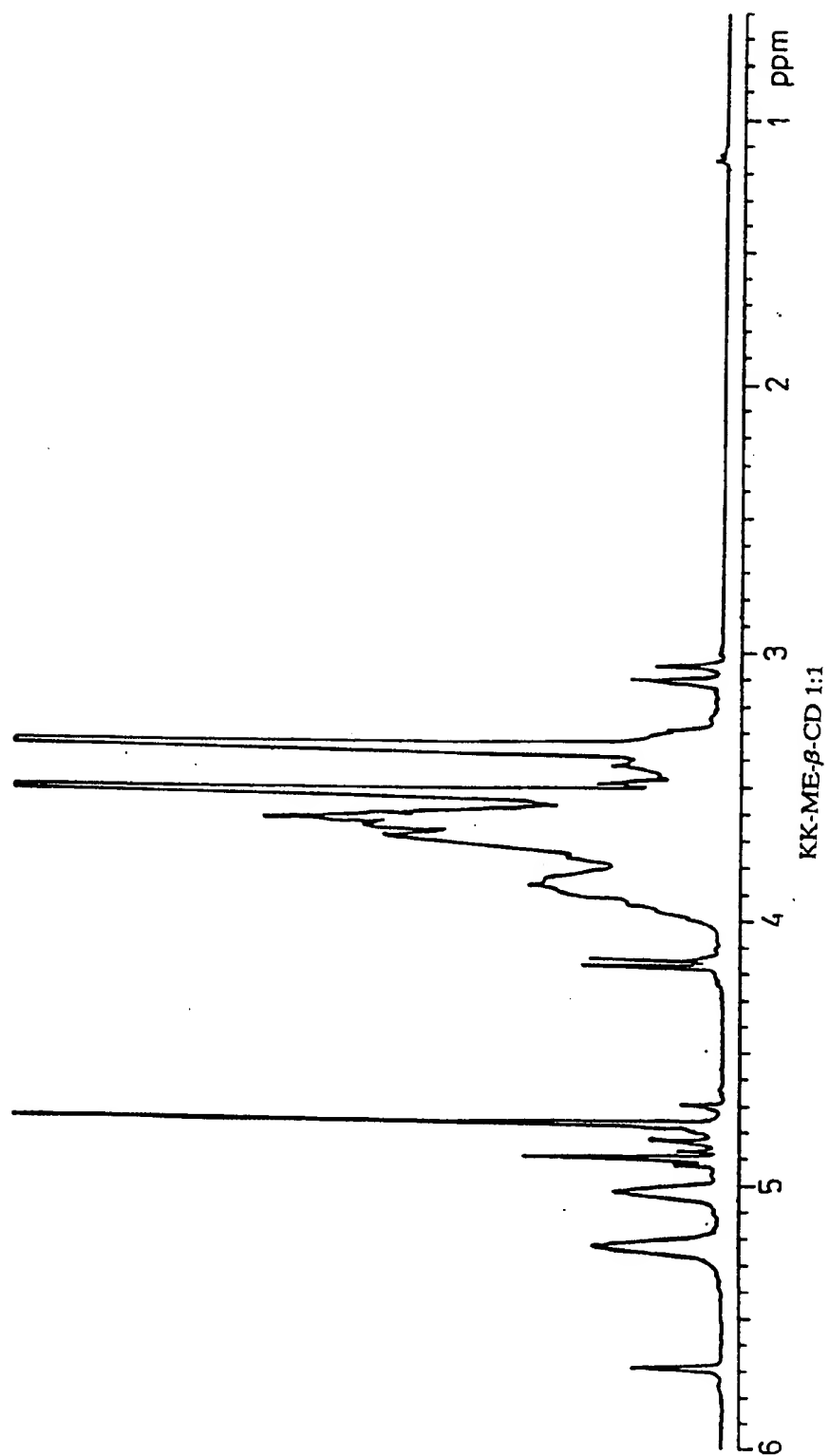


Fig. 1

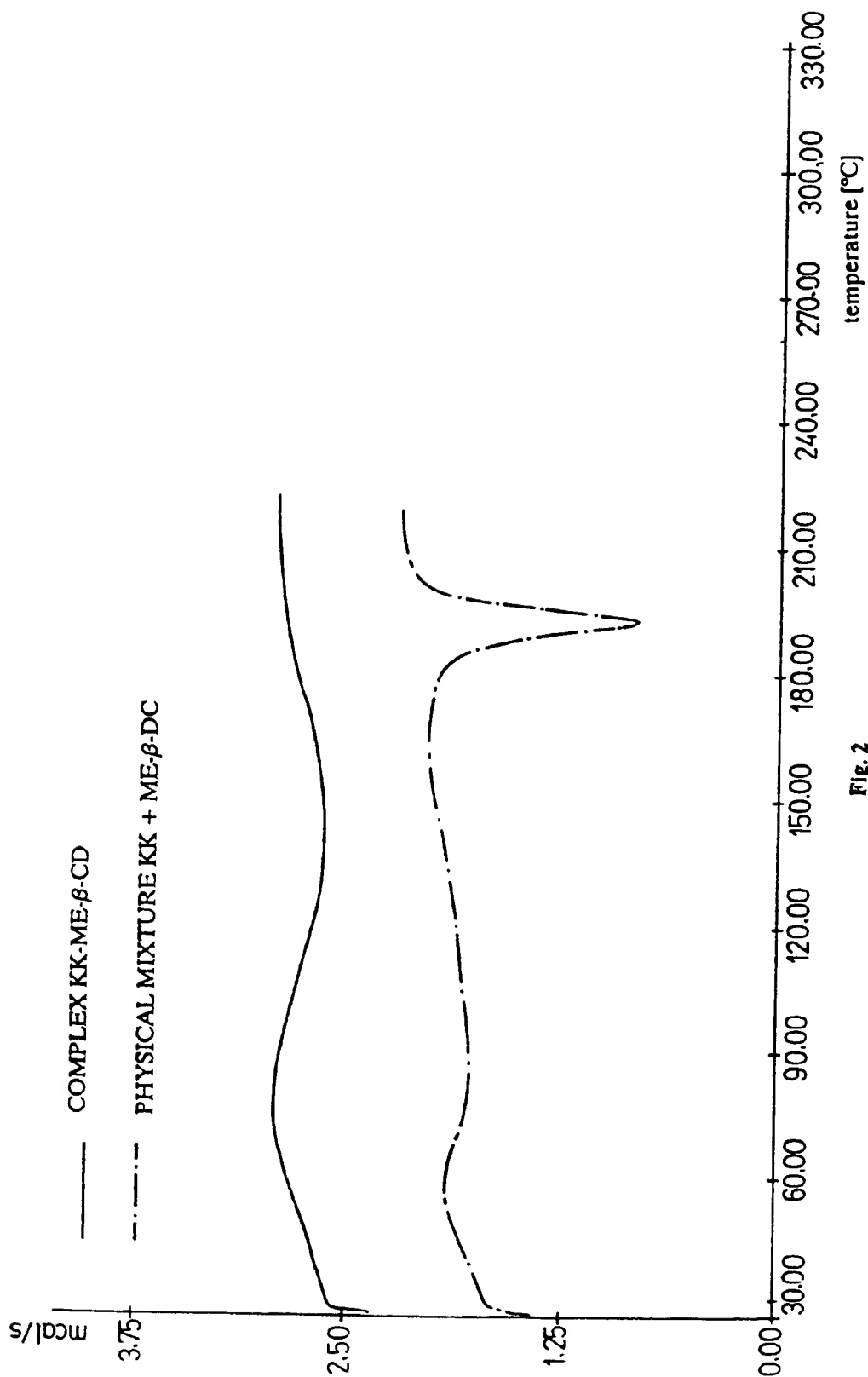


Fig. 2

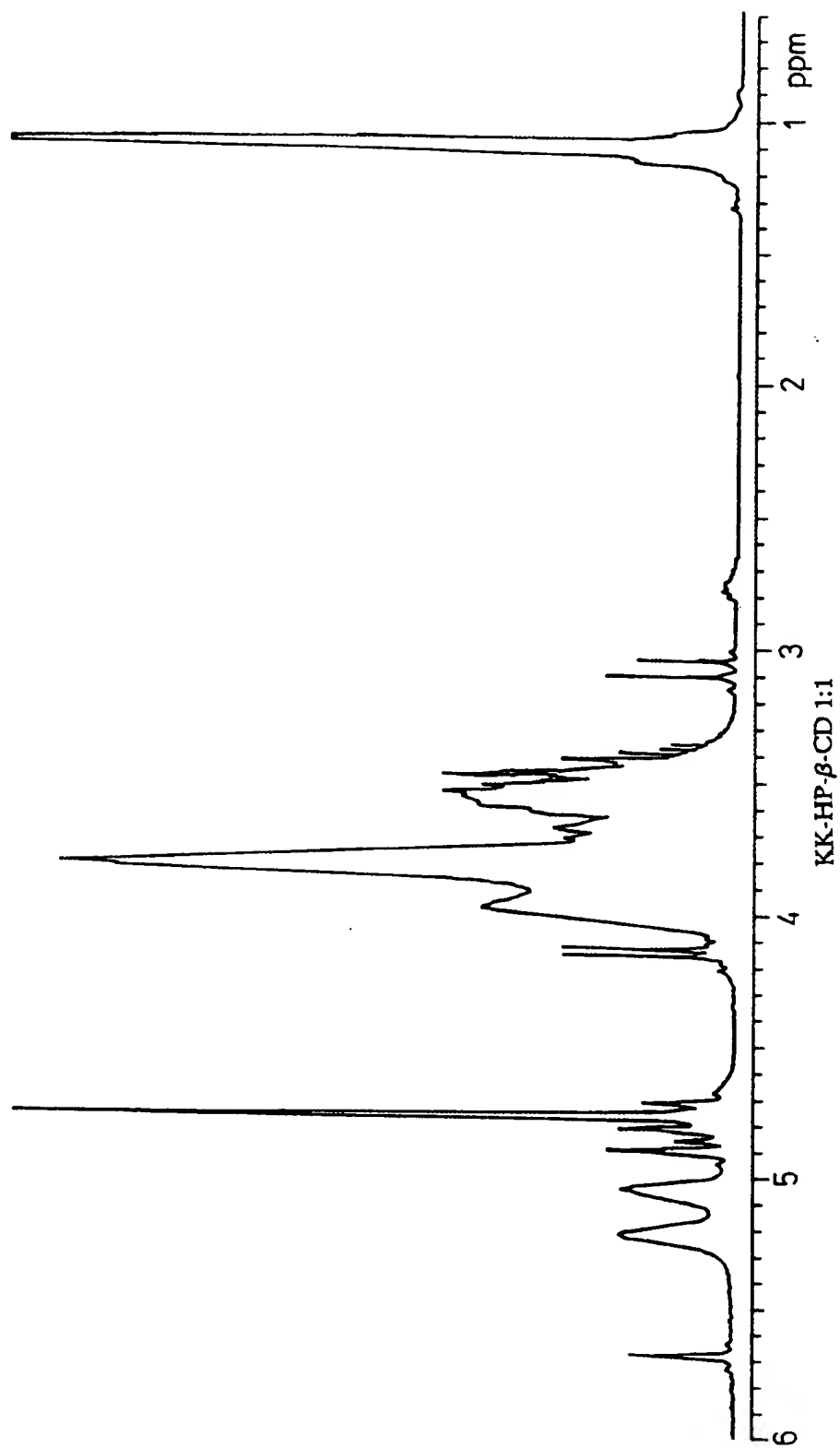


Fig. 3

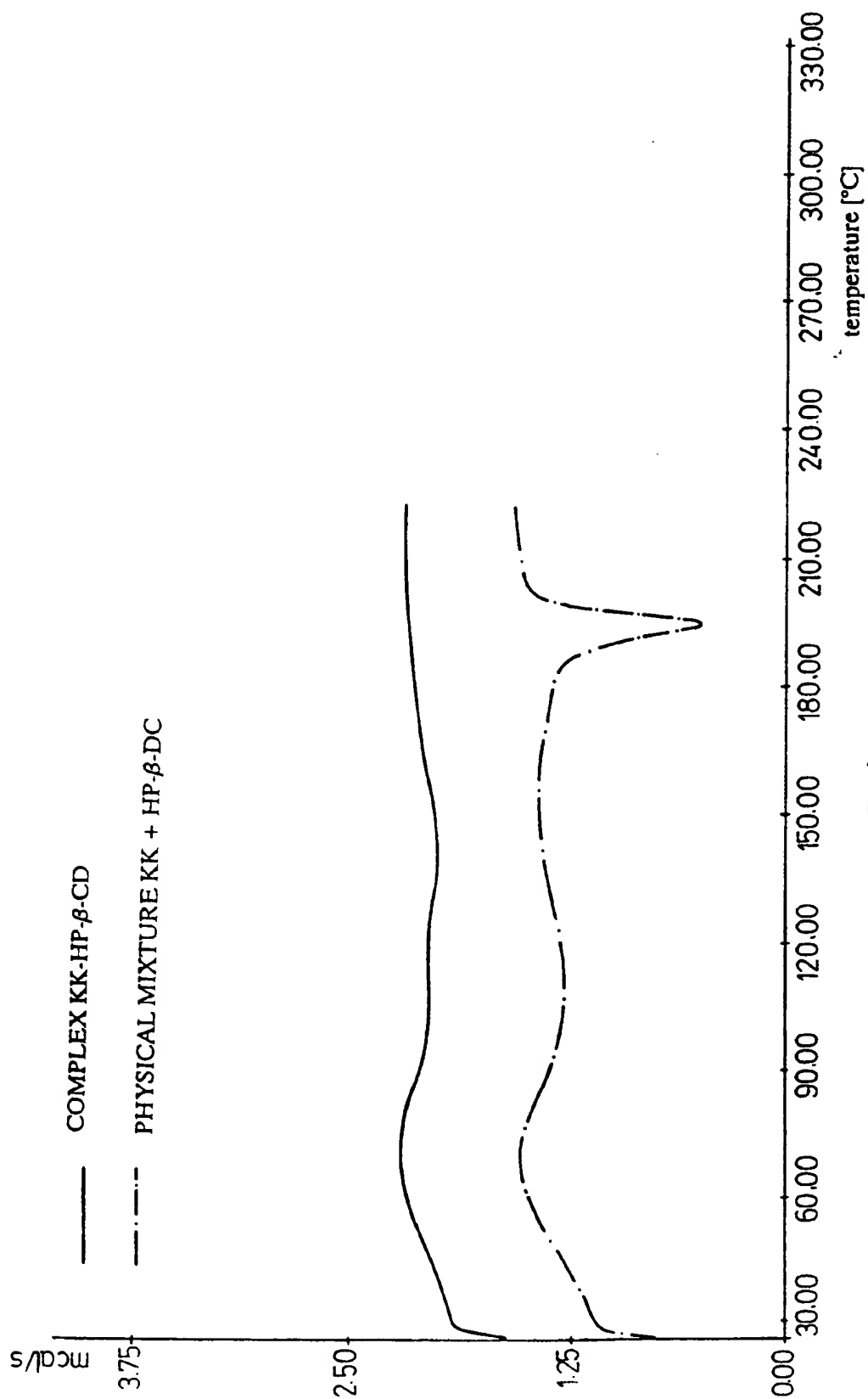


Fig. 4



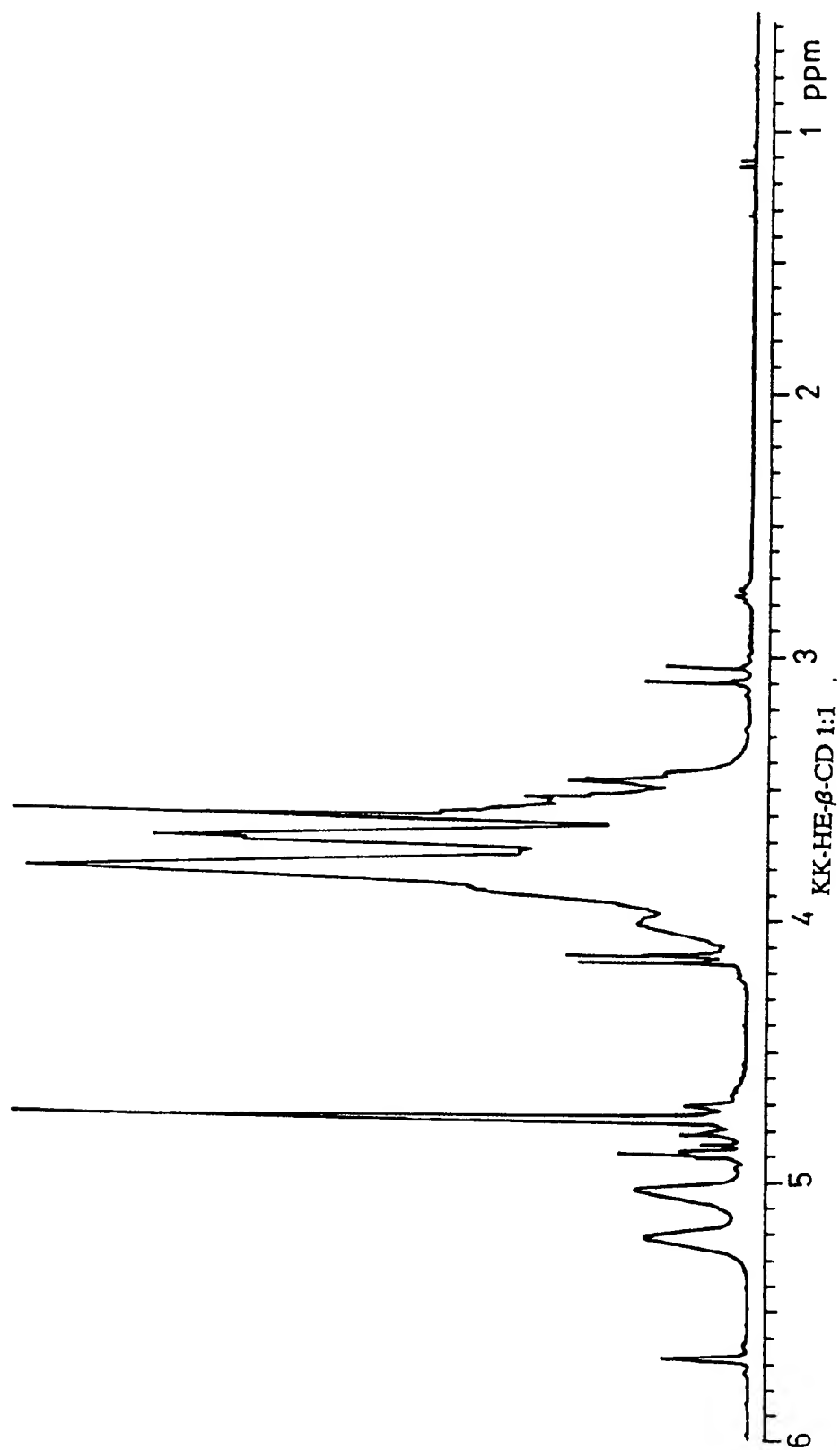


Fig. 5

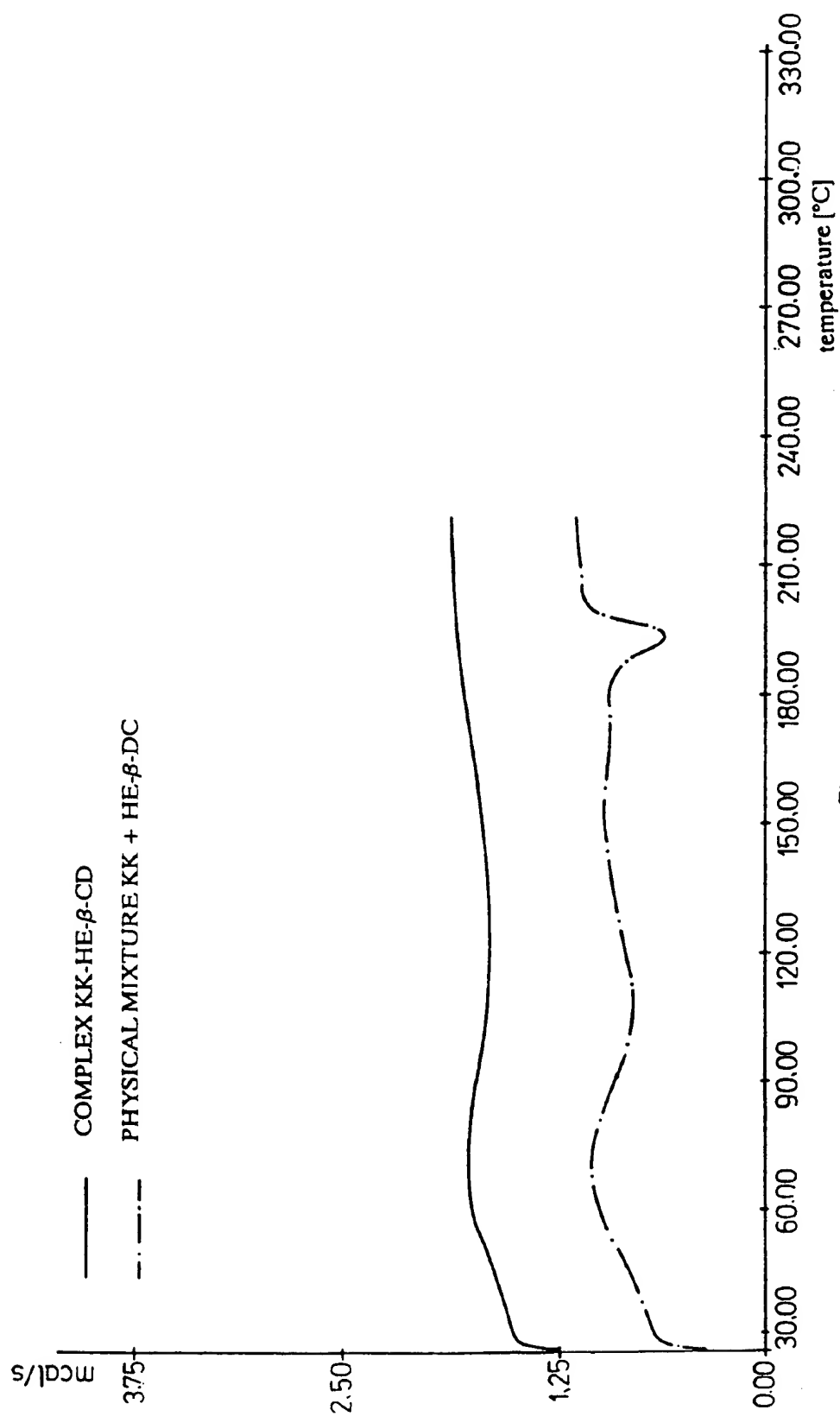


Fig. 6

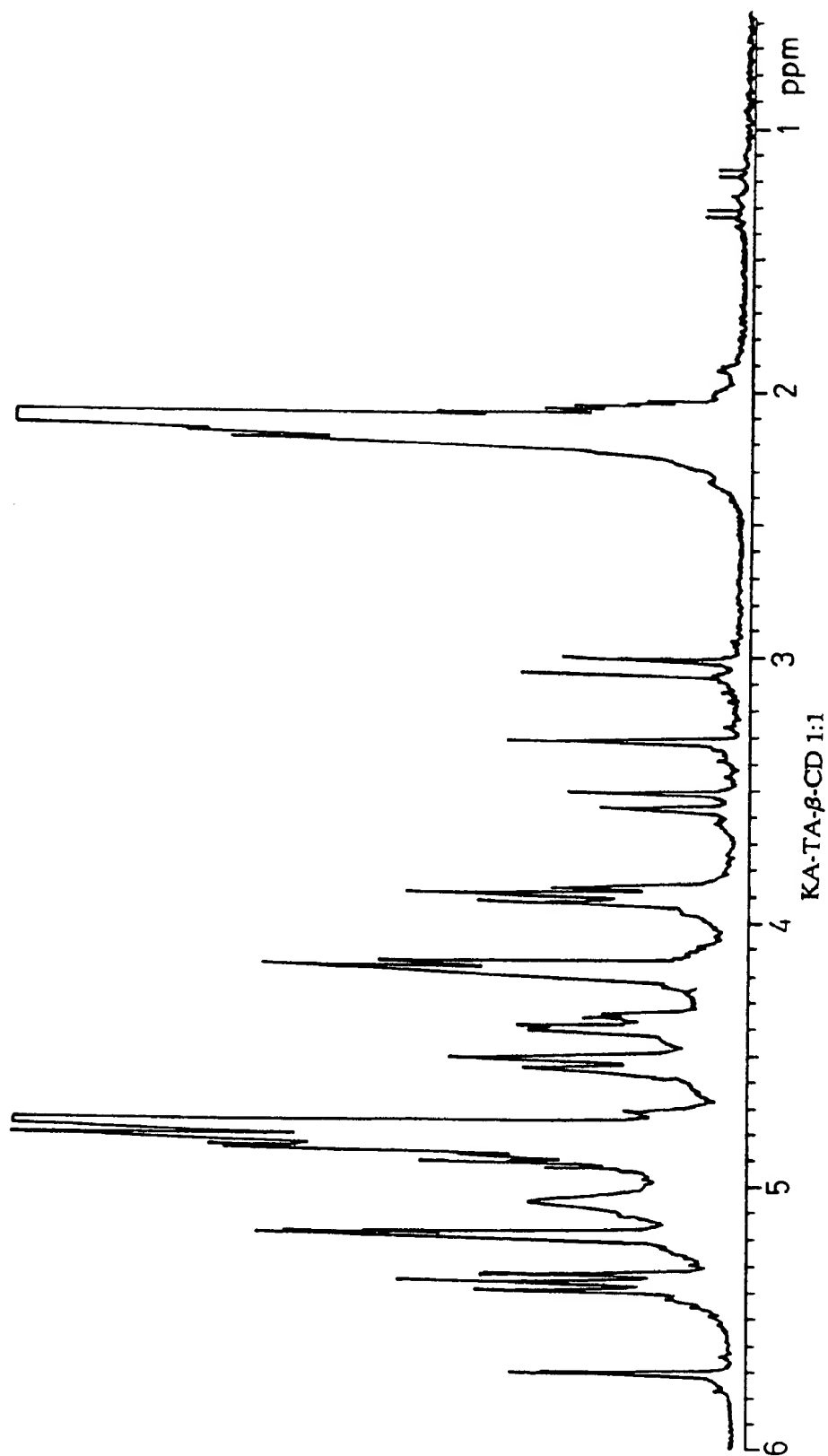


Fig. 7

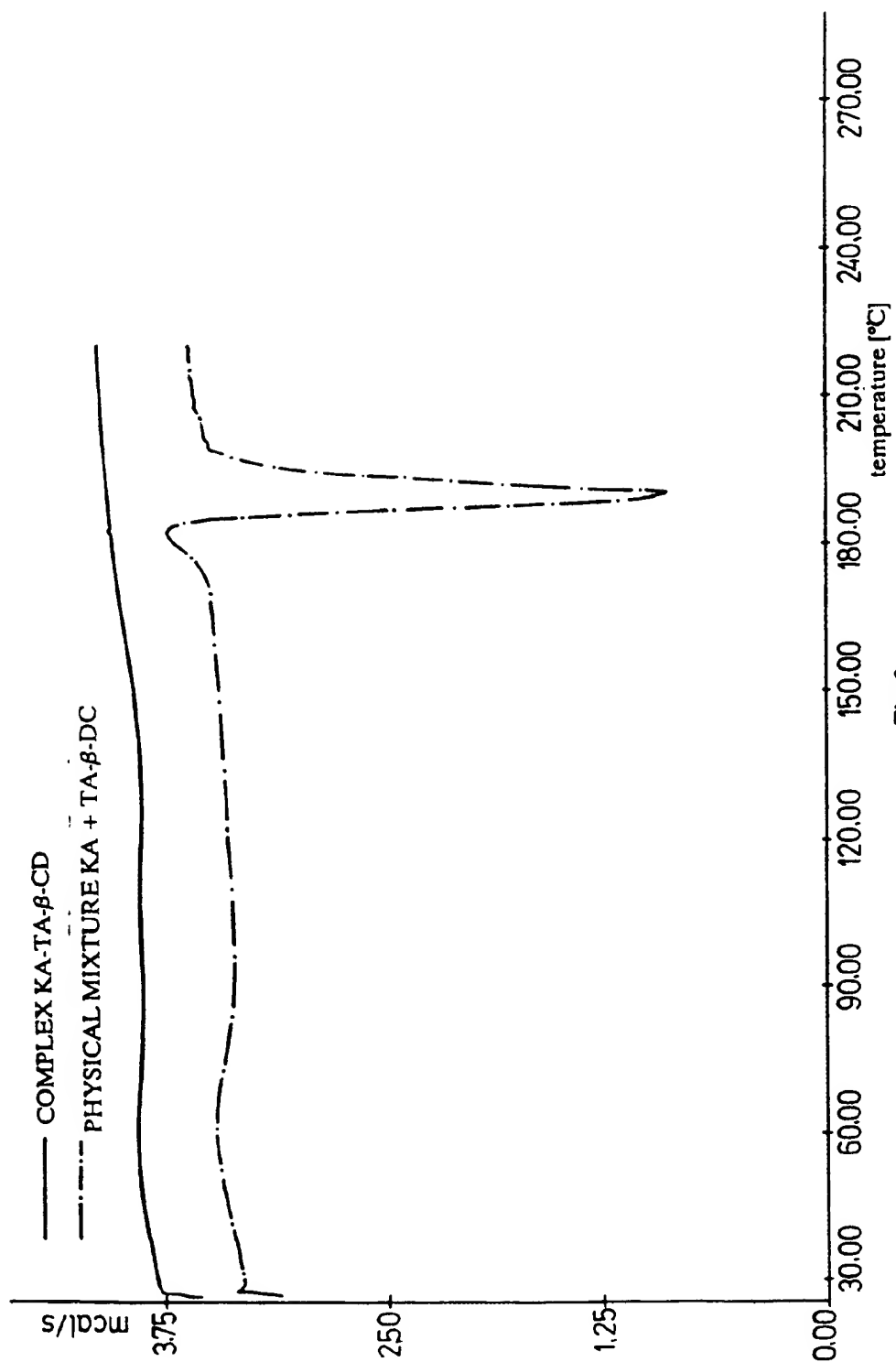


Fig. 8



European Patent  
Office

# PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

EP 93 11 0883

| DOCUMENTS CONSIDERED TO BE RELEVANT   |   |  |   |
|---|---|--|---|
| Category  | Citation of document with indication, where appropriate,<br>of relevant passages  | Relevant<br>to claim   | CLASSIFICATION OF THE<br>APPLICATION (Int. Cl. 5) |
| Y   | GB-A-1 589 917 (BEECHAM GROUP LTD.)<br>20 May 1981<br>* claims *  | 1-20   | A61K47/48<br>A61K31/42                            |
| Y   | EP-A-0 163 433 (TAKEDA CHEMICAL INDUSTRIES<br>LTD.)<br>4 December 1985<br>* claims *                                      | 1-20   |   |
| D,Y   | EP-A-0 293 537 (NISSHIN FLOUR MILLING<br>CO., LTD.)<br>7 December 1988<br>* page 4, line 36; claims *<br>& US-A-4 869 904 | 1-20   |   |
| A   | EP-A-0 010 904 (BEECHAM GROUP LTD.)<br>14 May 1980<br>* the whole document *  | 1-20   |   |
| Y   | EP-A-0 080 862 (BEECHAM GROUP PLC)<br>8 June 1983<br>* claims *   | 1-20   |   |
|   |   |  | TECHNICAL FIELDS<br>SEARCHED (Int. Cl. 5)         |
|   |   |  | A61K<br>C07D                                      |
| INCOMPLETE SEARCH   |   |  |   |
| <p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely:<br/>Claims searched incompletely:<br/>Claims not searched:<br/>Reason for the limitation of the search:</p> <p>see sheet C</p> |   |  |   |
| Place of search<br>THE HAGUE  |   | Date of completion of the search<br>14 SEPTEMBER 1993  | Searcher<br>BERTE M.J.                            |
| CATEGORY OF CITED DOCUMENTS   |   |  |   |
| X : particularly relevant if taken alone<br>Y : particularly relevant if combined with another document of the same category<br>A : technological background<br>O : non-written disclosure<br>P : intermediate document   |   | Y : theory or principle underlying the invention<br>E : earlier patent document, but published on, or after the filing date<br>D : document cited in the application<br>L : document cited for other reasons<br>A : member of the same patent family, corresponding document |   |

EPF FORM 1500 (12/82) (page 1)



EP 93 11 0883 -C-

INCOMPLETE SEARCH

Claims searched completely : 1-19  
Claim searched incompletely: 20

Remark : Although claim 20 is directed to a method of treatment of (diagnostic method practised on) the human/animal body (Article 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/ composition.